RESEARCH NOTE
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SEED FORMATION ON INDUCED HAPLOID PLANT AND CYTOLOGY OF ANTHER CALLUS FROM HYBRID RICE[12]

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The induction of six rice plants from anther callus of the cross, IR-8 (*indica*) × CN242-d_3 (*japonica*) was previously reported by Woo and Tung (1972). All of them were diploid. One haploid plant was also obtaind in 1971 from the same material by the same method. Phenotypes of the diploid plants differed slightly among themselves but significantly so from the haploid one. We have since conducted the progeny study of the plants and cytological analysis of the anther callus from which the aforementioned plants were derived.

The six diploid plants were grown to maturity but only five of them produced seeds. The seed producers were much taller than either parent, and all carried 30-50% sterility. Seeds were collected separately from the plants and grown for the next generation. Phenotypes of the progenies were found different in culm length, number of tillers, kernel shape, and growth habit within a single parental line and among lines. Culm lengths of the progenies were close to those of F_1 plants, and were much taller than the parental lines (Table 1) Variance of the character was highly significant compared with that of the mid-parent calculated in F-test. The tillers of progenies were less than those of the parental lines and F_1 plants, but statistically insignificant however. The phenotypic segregation thus reveals that the plants were most likely derived from the somatic tissue of anthers rather than from pollens. The genotype of those plants should be identical inasmuch as they were induced from somatic tissue of anthers, and would have no difference with those of F_1 plants. They, therefore, must be heterozygous carrying two different genes of semidwarfness located at different loci. The phenotypic difference among the induced plants would be caused by the recovery of

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regular cytoplasm with maternal composition during the differentiation of plantlets from callus tissue. The difference in the recovery may be carried over to the next generation and resulted in variation.

Table 1. *Phenotypic variations of plant height and number of tillers in progenies and parental varieties of the induced plants*

Parents	Culm length cm Mean S.E.	F Value	Tillers Mean S.E.	F Value
IR-8	82.87±2.38		28.00±2.77	
CN242-d_3	81.35±2.09		17.28±2.35	
F_1	120.57±2.64		14.14±2.17	
Progenies				
1	110.20±4.23	3.71**	11.54±2.10	0.70
2	102.83±4.27	3.81**	11.92±2.12	0.71
3	107.32±4.35	3.96**	13.50±2.44	0.95
4	122.20±3.82	3.05**	11.93±2.20	0.77
5	108.35±4.32	3.92**	13.00±2.40	0.92

The last one differentiated from callus tissue in spring, 1972, differed from the other five in its semidwarf stature and full sterility. However, one seed was obtained when it was hybridized with variety IR-22. This indicates that the megaspores were viable, and the sterility was due to pollen failure. These two distinct characteristics reveal that this plant was derived from pollen. The semidwarf stature was conditioned by a semidwarf gene from one of the parents, and its chromosome number was doubled during callus development. The male sterility of this plant was probably produced by the interaction of nucleus and cytoplasm of paternal origin.

The haploid plant obtained from the same cross in 1971 was treated with 0.5% colchicine in order to induce diploid progeny. The haploid plant was sterile; no seed has over been developed. However fourteen seeds from a panicle were successfully recovered from the treated plant. They appeared to be smaller than those of their parental varieties (Figure 1). The seeds are expected to yield a homozygous line derived from an intersubspecific cross of *indica* and *japonica* rice. This result is comparable with those of Burk (1970) who doubled a desirable haploid plant to an isogenic tabacco. The system employed here demonstrated that rice breeding by the recombination of desirable genotypes from distantly related speices can be prospected.

Young calluses from anthers of the F_1 plants and haploid plant mentioned

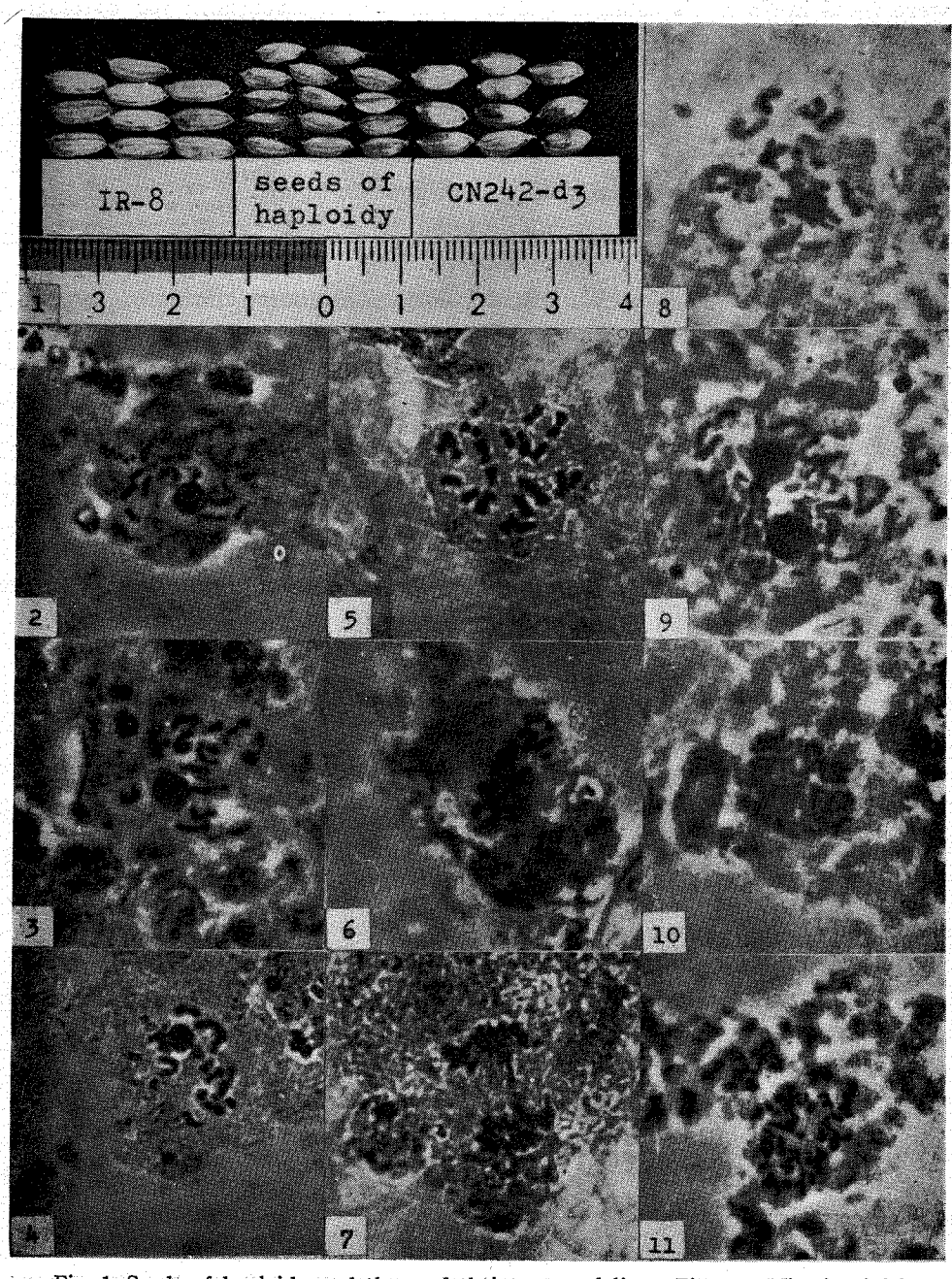

Fig. 1. Seeds of haploidy and those of their parental lines. Fig. 2-7. Mitotic divisions of anther callus from haploid plant. ×900. Fig. 2. Multi-contractions of chromosomes. Fig. 3-4. Prophase with 18 & 24 chromosome. Fig. 5-6. Metaphase with 21 & 18 chromosomes. Fig. 7. Anaphase with two laggard chromosomes. Fig. 8-11. Chromosome number of callus cells from F_1 anthers. Fig. 8-9. Prophase with 24 & 48 chromosomes. Fig. 10-11. Metaphase with 48 & 72 chromosomes.

were fixed in Farmer's solution and smeared with acetocarmine for cytological examination. Calluses having euploid cells of haploidy and diploidy (12 and 24 chromosomes) and aneuploid cells were found from anthers of haploid plants (Figures 2–7). Pollens of the haploid plants generally do not carry the complete genetic constitution, and do not theoretically survive in culture. Therefore the callus tissue induced was of somatic origin.

Callus cells of F_1 plants (2n=24) varied widely in chromosome number from diploidy to hexaploidy (Figures 8–11). They may generate plants of different polyploidy. This mechanism of induction has been elucidated by Niizeki and Grant (1971) who produced tetra- and octoploid tobaccoes from callus. The callus induced from F_1 anthers may differ in their origin with those of haploid plant. The pollens of diploid plants have carried a complete set of chromosomes and the induction of haploid plants has been reported by Woo and Tung (1972) and by Niizeki and Oono (1968). Pollens of haploid plant are nonviable; nevertheless the somatic cells have a complete set of chromosomes, which may initiate a homozygous progeny. Similar result was reported by Watanabe *et al.* (1973). They found that the calluses of *Chrysanthemum* were mostly derived from the connective tissue between thecae of the anthers.

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單倍體水稻的種子誘導和雜種花葯Callus細胞的染色體

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前文報導誘自雜種花葯的六株水稻，其中五株成長後形態略有差別，均有30—50%不孕性，第二代形態分離明顯，足以證明該五植株係導自花葯的體細胞；第六株矮生不孕，乃誘自花粉細胞，其矮生基因來自親本之一。得自相同材料的一株單倍體，用秋水仙精0.5%處理，在一穗中得種子14粒，現已成長爲植株，其外表型一致，極可能是純系。第一代雜種的花葯Callus，染色體的數目由24至72不等，單倍體稻的則爲12至24染色體。